SEED COAT ANATOMY AND TAXONOMY IN *EUCALYPTUS*. II.*

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(With Plates xi–xiii; Text-figures 20–39.)

[Read 26th August, 1959.]

Synopsis.

Blakely's Series Eudesmiae of the Section Macrantherae comprises 12 species of which 11 have been examined. Except the Subseries Leptospermae their seeds are of hemitropous structure, bifacial, the dorsal side uniform, the ventral side containing chalaza (with the vascular system), hilum and micropyle. There is an immediate transition of the conducting tissue from the placenta to the chalaza, that is to say without the formation of a raphe. The inner epidermis of the outer integument is a crystal epithelium par excellence, the inner integument is suberized, the inner cuticle very tender and smooth (or with inconspicuous projections).

Apart from these common features the separate species often display considerable differences not only in morphological aspects (seed smooth, ribbed or winged) but particularly with regard to the anatomy of epidermis, hilum, micropyle etc., suggesting some necessary rearrangement within the Eudesmiae or even a transfer of some species to other groups of the Macrantherae (or the reverse).

The Subseries Leptospermae (*E. tenuipes* and *E. curtisii*) exhibits—as far as the testa structure is concerned—no relation to the hemitropous Eudesmiae. The seeds are anatropous with a typical raphe, hence with a different relative position of chalaza, hilum and micropyle and a different vascularization. The crystal epithelium is missing, the inner cuticle rather conspicuous and with relatively prominent ribs. There is no doubt from the complete uniformity in testa structure that these two species are very close relatives and sharply demarcated from the remainder of the Eudesmiae.

Section MACRANTHERAE. Series Eudesmiae (Benth.) Blakely.

Introduction.

Of the twelve species listed by Blakely in the Series Eudesmiae the seed of the following eleven were examined.

(1) Subseries Fasciculares: *E. erythrocorys*, *E. tetragona*, *E. eudesmoides*, *E. ebbanoensis*.

Subseries Efasciculares: *E. odontocarpa*, *E. tetrodonta*.

Subseries Holocalyces: *E. lirata*, *E. similis*, *E. baileyana*.

Their seeds are of hemitropous structure and we distinguish between the ventral side (with chalaza and its vascular system, hilum and micropyle) and the uniform dorsal side.

(2) Subseries Leptospermae: *E. tenuipes* and *E. curtisii*.

Their seeds are of anatropous origin and we may distinguish between a raphe side (with chalaza and raphe bundle) and a uniform raphe-free side. Hilum and micropyle lie side by side at the basal end of the seed.

The following explains the lettering in the Text-figures and Plates: *cd*, clinging disc; *ch*, chalaza; *cot*, cotyledons; *cr*, crystal epithelium; *ec*, embryo cuticle; *end*, endosperm; *h*, hilum; *hy*, hypocotyl; *ic*, inner cuticle; *ie*, inner epidermis; *ii*, inner integument; *lc*, lignified cells; *m*, micropyle; *mu*, mucilage; *nr*, nucellus remnants; *oc*, outer cuticle; *oe*, outer epidermis; *oi*, outer integument; *rm*, root meristem; *vb*, vascular bundle.

In the following the different parts of the testa are described together with some remarks on the nucellus, endosperm and embryo.

Outer Integument.

Originally a transitory storage tissue supplying the growing seed with food material, it undergoes during maturation various alterations in contents and structure of its cells. The final stage which is discussed here is that reached in ripe abscissed seed.

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* See Part I, Proc. Linn. Soc. N.S.W., 1958, 83, Part 1: 20–32.

Outer Epidermis.

The somewhat tabular cells are polygonal in surface view and more or less iso-diametric (Fig. 20, 21), the only exception being *E. erythrocorys* where most of the cells are elongated parallel with the surface (Fig. 22). The outer walls are thin, giving cellulose reaction (*E. eudesmoides*, *E. baileyana*, *E similis*), or thick and lignified (*E. erythrocorys*) or have mucilage as secondary wall deposits (*E. tetragona, E. ebbanoensis*). The mucilaginous cells of *E. odontocarpa* are of particular interest because of the complex structure of the outer wall. The smaller cells have a mucilaginous layer beneath a thin outer wall of cellulose, outlined against the lumen by a tender cellulose membrane (Fig. 23*a*). In the large bulging cells there is next to the lumen a thick, lignified and pitted membrane (Fig. 23*b*). In some instances this membrane was seen embedded within the mucilage of which the inner layer showed traces of pits continuous with those of the lignified membrane (Fig. 23*c*). This suggests that a formerly solid wall underwent *pro parte* a subsequent mucilaginous modification. In *E. tenuipes* and *E. curtisii* not only all epidermal cells have membrane mucilage but also many cells of the integumentary parenchyma (Fig. 24). The mucilage is impregnated with brown tannin-like substances. After their removal with Eau de Javelle it stains blue with chloroiodide of zinc. Cuprammonia stains for a short while light blue, whereupon, swelling rapidly, the mucilage exhibits striking lamellations before being dissolved.

Phlobaphene deposits in epidermis cells are a regular feature in the anatropous Eudesmiae (*E. tenuipes*, *E. curtisii*), recalling the anatropous Renantherae.

Where the outer walls are thin they have collapsed in ripe seed and are more or less appressed to the inner walls rendering the surface minutely pitted (*E. baileyana, E. similis*). On the other hand a gregarious occurrence of thick-walled bulging cells causes the wrinkled appearance (*E. odontocarpa*). In *E. tetrodonta* the sometimes warty surface is due to groups of lignified cells elongated perpendicularly to it.

The cuticle covering this epidermis is well preserved in *E. erythrocorys* and *E. tenuipes*, but in *E. baileyana* the usual stains and reagents failed to reveal its presence. In some sections of *E. eudesmoides* (and some other species) the cuticle was seen as an unbroken line, in others only in fragments, more or less detached from the epidermis, or, if not entirely missing, merely as a dotted line. Of course, the ovule always has a perfect cuticle which during maturation to seed may become disorganized or even completely resorbed. A loosening of its contact with the epidermis may also occur so that sectioning followed by treatment with reagents may tear off and wash away what remained of it.

Inner Epidermis.

This epidermis is, in all seeds of hemitropous structure, a typical crystal epithelium, each cell containing one large and a few smaller crystals of monoclinic calcium oxalate monohydrate. Crystalline granules (crystal-sand) are also often present, some-times also elongated crystals (styloids).

The most common well-developed crystals are six or eight-faced, more rarely ten-faced. Professor Machatschki* (in litt.) gives the following indices which we reproduce with his sketches (Text-fig. 25).

Hence we may infer that Figure 25*a* is a combination of the prism (110) and the basal pinacoid (001), Figure 25*b* shows an additional clinopinacoid (010) and finally the ten-faced crystal in Figure 25*c* also the orthopinacoid (100).

The larger crystals are encased within cellulose sheaths which, after dissolving the crystals, imitate so precisely their outline that only with a polarization microscope are these structures recognized as merely empty forms.

The cells are closely packed, their morphologic outer walls thick, constricting the lumen to such extent that there remains very little room to accommodate the crystals (Fig. 28, 32). This may explain their tabular habit parallel to the basal pinacoid (001).

As part of the integument which develops from a more or less circular rim at the base of the future nucellus the crystal layer of the mature seed exhibits at its base a circular, elliptic or oval gap, the "window in the crystal armour"—a very appropriate designation.
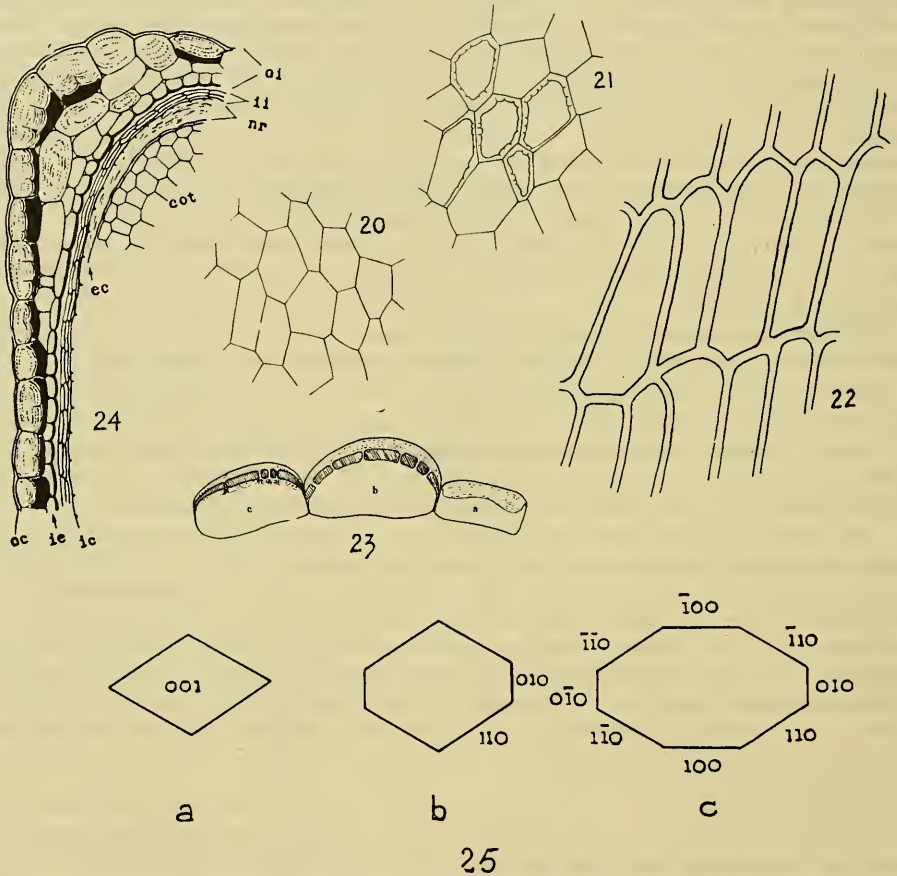
Text-fig. 20-24.—20-22, surface view of the dorsal epidermis, 100×.   20, *E. baileyana*; 21, *E. tetrodonta*, showing a group of sclerified cells; 22, *E. erythrocorys*.   23-24, mucilaginous epidermis cells.   23a-c, *E. odontocarpa*, three types of outer wall modifications, 160×; 24, *E. tenuipes*, transv. sect. through an edge of seed.   Mucilage dotted, phlobaphenes black, 250×

Text-fig. 25a-c.—*E. eudesmoides*, oxalate crystals of the inner epidermis (Crystal epithelium) of the outer integument.

The cells of this epidermis are crystal-bearing to its very fringe (*E. tetragona, E. eudesmoides, E. baileyana*) or some rows bordering the "window" are empty (*E. odontocarpa*) and moreover both empty and suberized in *E. erythrocorys* and *E. ebbanoensis*. The border is more or less smooth or as in *E. similis* jagged (Fig. 35b, c).

This striking crystal epithelium in hemitropous seed is missing in the anatropous Subseries of Leptospermae (*E. tenuipes* and *E. curtisii*). Crystals occur here, too, but only dispersed in the hilar and chalazal region and along the raphe, the cells having

only slightly thickened outer walls (Fig. 24 *ie*). The far greater part of the inner epidermis has no crystals.

Integumentary Parenchyma.

The parenchymatous tissue between the two epidermal layers and originating from them is always multi-layered on the ventral or raphe side. From here the number of layers gradually decreases towards the opposite side where it is reduced to a few (*E. erythrocorys, E. tetragona*) or to a single, often interrupted one (as in most of the species), and sometimes even missing (*E. baileyana, E. lirata*). A greater amount of this parenchyma is also found in wings (Fig. 27, 28) and in the costae.
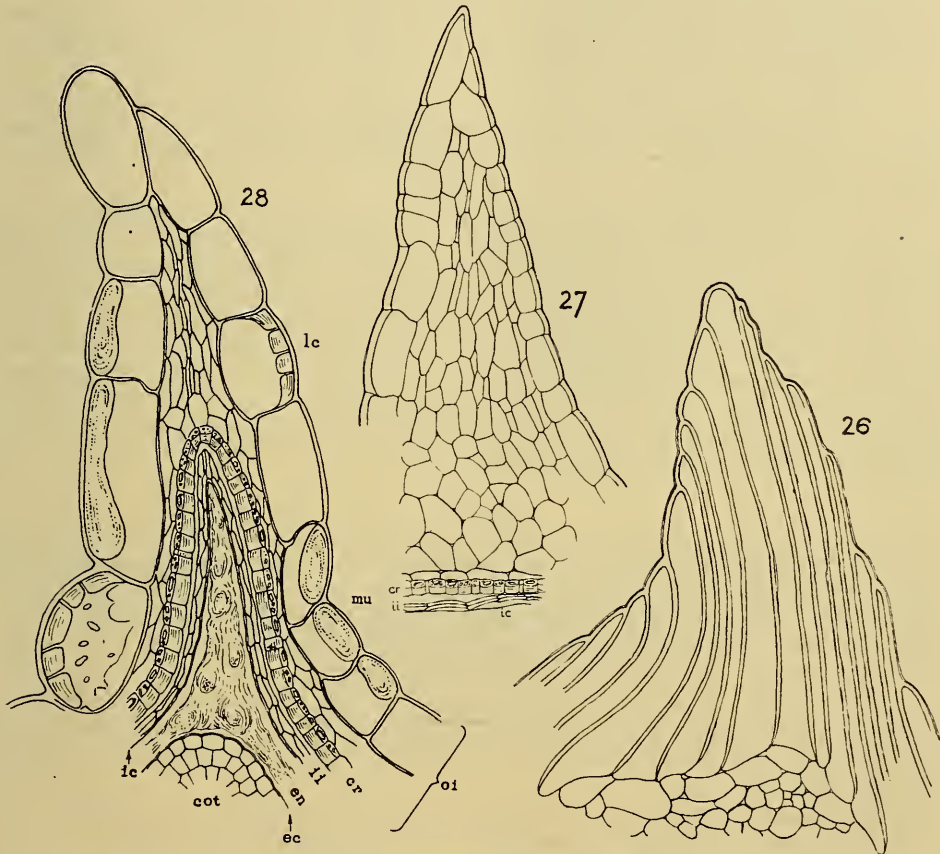
Text-fig. 26–28.—Transv. sect. through ridges and wings. 26, *E. erythrocorys,* ridge, 50×; 27, *E. tetragona,* wing, 130×; 28, *E. odontocarpa,* wing, 250×.

The homogeneity of this tissue is often disturbed by the occurrence of cells—single or in groups—with different contents or wall structure. Crystal idioblasts are for instance very frequent in the ventral side of *E. ebbanoensis*, with crystals partly embedded in the uneven wall thickenings (Pl. xi, fig. 5*b*), in the micropylar region of *E. erythrocorys* (Pl. xi, fig. 1*a*), in wings of *E. tetragona*, etc. Sclereid idioblasts are not infrequent in *E. erythrocorys*, in *E. tetrodonta* adjacent to the inner epidermis and in *E. ebbanoensis* among suberized cells below the hilum (Pl. xi, fig. 5*b*). In *E. erythrocorys* suberized cells along the bundle branches were observed. Mucilaginous cells occur in *E. tenuipes* (Fig. 24) and *E. curtisii*. Phlobaphene deposits are often seen (*E. tetrodonta, E. tetragona*) and impregnation of all walls with tannins, including those of both epidermal layers, is the rule. Because of this the seed coat is of dark (brown to black) appearance.

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INNER INTEGUMENT.

This is always two-layered and suberized. The cells are tabular, without inter-cellular spaces, empty and very often completely obliterated. Like the outer integument it embraces with its circular base the chalaza. This is true also of its inner cuticle which is always present though not as conspicuous as in the Renantherae.* However, the median cuticle, separating the two integuments and present in the ovule, is missing in mature seeds. Only in *E. tetrodonta* were some fragments of it seen.

RIDGES AND WINGS.

The seeds of *E. baileyana*, *E. similis*, *E. lirata* and *E. ebbanoensis* have a smooth surface, that is to say their testa is not expanded into ridges or wings.

The angular seeds of *E. erythrocorys* have prominent ridges built up by epidermis cells exceedingly elongated at right angle to the surface (Fig. 26). The ridges in *E. tetrodonta* are of similar structure.

E. tetragona has wings, the broadest among the Eudesmiae, though scarcely exceeding 1¾ mm. in width. The cross section through the circumferential wing reveals that the outer epidermis and parenchyma of the outer integument are the constituent parts (Fig. 27).

E. eudesmoides and *E. odontocarpa* have a circumferential membranous wing separating the ventral side from the dorsal. This delicate and narrow expansion has nevertheless a complex structure as both integuments and endosperm tissue (or in the vicinity of the chalaza, nucellar tissue) participate in its build up. The presence of the thick-walled crystal layer confers some mechanical protection which in *E. odonto-carpa* is increased by lignification of some epidermal cells (Fig. 28). During germination the wing at the root pole is neatly split by the pressure of the swelling clinging disc, whereupon the hypocotyl emerges between the wing halves. Therefore the role of the clinging disc is not only to fix the germinating seed to the substratum, but as it expands by growth to burst the testa first of all.

HILUM.

After the shedding of the seed an elliptical, more or less discernible scar, the hilum, marks the abscission region. In hemitropous species it is located on the ventral side, overlapping partly the chalaza, though sometimes but to a very small extent (*E. eudesmoides*). However, the vascular bundle always has direct access to the chalaza. Therefore, there is no "raphe" so characteristic of the anatropous seed (*E. tenuipes* and *E. curtisii*).

In *E. baileyana*, *E. tetragona*, *E. eudesmoides* and *E. similis* the hilum is bordered by thin-walled peripherally elongated cells forming a sort of parenchymatous sheath (Fig. 29, 30). In *E. erythrocorys* the hilum is sharply circumscribed by a rim of sclereids (Fig. 31).

The surface of the hilum is flat or a shallow depression (*E. baileyana*, *E. eudesmoides*, etc.) or convex and shield-like (*E. ebbanoensis*, Pl. xi, fig. 5b).

Three types of cicatrization of the hilum were observed:

(1) Desiccation, shrinkage and collapse of the exposed cells, giving the hilum a pulverulent appearance, as for instance in *E. tetragona*, *E. eudesmoides*, *E. similis*. *E. erythrocorys* (Fig. 31). There is no suberization of the exposed cells and even cutinization was not traceable. Therefore, this is a very primitive type of wound healing.

(2) In *E. odontocarpa* the hilar scar is sealed by sclereids with lignified walls impregnated with tannins. These sclereids are either closely packed (Pl. xii, fig. 1) or dispersed in groups, and belong not only to the uppermost layer but also to the subjacent tissue (Fig. 32). It must be pointed out, however, that the sclerification seldom extends over the entire hilum, but is mostly restricted to that part which lies

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* In some instances where this cuticle was very delicate and the usual stains were not adequate we used successfully a Rosanilin solution discoloured with SO_2.
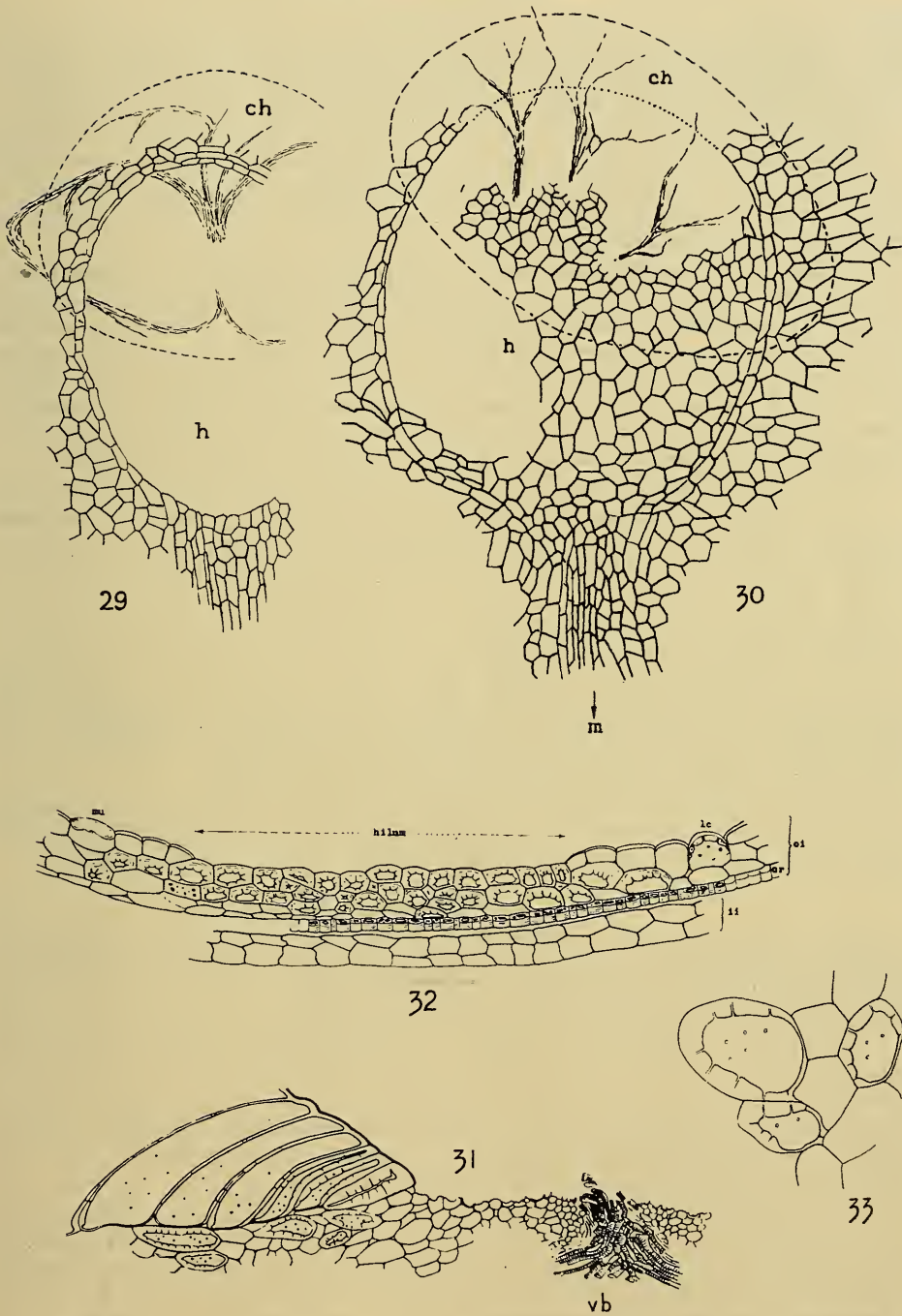
Text-fig. 29-33 (hilar structures).—29-30, surface view of the hilum (*h*) overlapping the chalaza (*ch*). Some tissue partly removed to show the extent of the chalaza (broken line) and the vascularization. 29, *E. baileyana,* 50×; 30, *E. tetragona,* 65×, three bundle strands enter separately into the chalaza. The arrow points in the direction of the micropyle *m.* 31-32, trans. sect. through the hilum. 31, *E. erythrocorys,* 100×. A rim of macrosclereids surrounds the hilum in which a vascular bundle (*vb*) enters; 32, *E. odontocarpa,* 160×. The hilum is sealed by sclereids (metaplasy by sclerification). 33, *E. erythrocorys,* surface view of the hilum with dispersed sclereids, 200×.

outside the chalaza (Pl. xii, fig. 1). Thus the portion overlapping the chalaza was often seen without sclerification but sometimes bordered by a more or less complete rim of thick-walled cells. This form of cicatrization represents, at least partially, a case of metaplasy* by sclerification and wall impregnation by antiseptic substances.

Sclereids with lignified uneven wall thickenings can be seen also in the hilum of *E. erythrocorys* where they occur singly or in groups, variably dispersed among thin-walled cells (Fig. 33). Their occurrence is too scanty to be of any value for the protection of the scar, but unequal tensions in such a heterogeneous tissue may facilitate the final separation of the seed.

(3) The most perfect case of metaplasy, that is by suberization, is exhibited in *E. ebbanoensis* where all surface cells of the hilum are suberized and completely filled with solid phlobaphenes extremely resistant to acids and bases. Suberized cells occur also in the underlying layers of the hilar cushion, intermixed with crystal- and sclereid-idioblasts (Pl. xi, fig. 5a-b). This is a remarkable case because Netolitzky, who critically revised the entire literature on Angiosperm seeds (about 1,100 papers), states that hitherto (1926) no suberization has been observed on the hilum. Hintringer (1927), who examined 80 species of 9 families, confirms Netolitzky's statement.

It is worth mentioning that the agents stimulating the formation of sclereids (*E. odontocarpa*) or inducing suberization (*E. ebbanoensis*) bring about the same metaplasic changes in some epidermis cells adjacent to the hilum (Pl. xi, fig. 5a). Analogy can be drawn with the traumatic stimulus of wound hormones in tissues not directly affected by injuries.

In the hilum of *E. similis* suberization is confined to a few cells surrounding the entrance of the bundles into the chalaza.

As we are concerned only with the hilar structure of the mature abscissed seed the mechanism of its separation from the placenta and the time of the metaplasic wall modifications are not discussed. This would require ontogenetic investigations. But it seems quite certain that the metaplasy precedes the abscission.

Finally, the break in the vascular bundle occurs in the transition region between placenta and hilum where short tracheids have been preformed (Fig. 31). The somewhat protruding bundle stubs can be located on the hilum of many species.

Chalaza.

The crystal layer, the suberized inner integument and the inner cuticle form together at their base a "window" which embraces and delimits laterally in a spectacular way the inner part of the chalaza. The outer part (above the window) passes imperceptibly into the parenchyma of the outer integument.

Two features characterize the chalaza: (1) The presence of a suberized tissue, the so-called "chalaza cork", in mature seed closing the gap and filled with dark coloured phlobaphenes. Thus, in some instances the chalaza is externally discernible without optical aid as a dark spot, in particular in those cases where these deposits are confined not only to the suberized part but occur throughout the whole chalaza. (2) The presence of the conducting tissue above the suberized zone.

As in the Renantherae the nucellus tissue† can participate in a variable amount in the formation of the chalaza cork. In *E. baileyana* and *E. tetragona* it originates almost entirely from the nucellus, in *E. odontocarpa* it is chiefly the true chalaza parenchyma of which the inner part undergoes the suberization. *E. tetrodonta* is exceptional in having no chalaza cork.

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* The term "metaplasy" was introduced into plant anatomy by Küster (1903) to designate progressive changes in histological character other than by cell growth or division. In our case these changes concern chiefly a sclerification or a suberization to form a protective cicatrice over the abscission region.

† In sections through the chalaza an imaginary line closing the gap in the inner cuticle defines neatly the chalaza from the nucellus.

VASCULARIZATION.

The vascular supply to floral parts has received a great deal of attention in recent years. It has been suggested that the vascular bundles of an organ are more conservative than its external form and therefore they may contribute to the solution of phylogenetic and taxonomic problems. A critical review of the whole literature—*pro* and *contra*—was given by V. Puri (1951).

In the Eudesmiae with anatropous seed the vascular bundle is brought up from the hilum to the chalaza through the raphe (Fig. 34*a*, *b*).

In the Eudesmiae with hemitropous seed the conducting tissue enters directly from the placenta into the chalaza. In some species the number of strands penetrating the seed is not constant. In *E. tetragona* we saw one to three (Pl. xiii, fig. 2 and Text-fig. 30), in *E. similis* (Fig. 35*a*, *b*, *c*) up to four strands entering separately into the chalaza. A variable number can be seen also in *E. erythrocorys*, whereas in *E. eudesmoides* (Pl. xii, fig. 2), *E. ebbanoensis*, *E. baileyana* (Pl. xiii, fig. 1) and *E. lirata* (Fig. 36*a*, *b*) we observed always one single strand.

The bundle entrance never lies in the centre of the hilum but always close to its upper border. From here the bundle spreads immediately into several branches (*E. odontocarpa*, Pl. xii, fig. 1) or the ramification starts at a short distance from its entrance (*E. baileyana*, Fig. 29 and Pl. xiii, fig. 1; *E. tetrodonta*, Fig. 37*a*, *b*).

The whole venation system seldom lies within the chalaza, most frequently some of the stronger branches penetrate somewhat the parenchyma of the outer integument but without taking an intra-integumentary course.

In *E. odontocarpa* the nearly straight branches spread palmately in the chalaza (Pl. xii, fig. 1), whereas in other species they are curved in varying degrees, displaying, especially the outer ones, an arctuous course along the chalaza border, as for instance in *E. baileyana* (Pl. xiii, fig. 1). Even abrupt angular turns can be observed (Fig. 35).

The bundles are amphicribral, the tracheary elements being only tracheids with helically thickened walls. Veins and veinlets terminate either freely with a single or double file of tracheids, or some of the branches may merge at their tips, as for instance in *E. baileyana* (Fig. 29) or *E. similis* (Fig. 35). The latter has sometimes transverse anastomoses interconnecting main strands (Fig. 35*c*).

Considered as a whole the vascular supply is very poor in *E. tetragona* (Pl. xiii, fig. 2), whereas in *E. erythrocorys* it displays a high degree of ramification and, by merging of the branchlet endings, a reticulate venation.

The venation patterns as shown here for hemitropous seeds exhibit a relatively wide range of variation within this small Series, even allowing for the variations within the same species. Thus at first glance it seems doubtful whether any taxonomic conclusion can be reached at this stage before the investigation is extended to the remaining hemitropous Macrantherae.

MICROPYLE.

Its approximate position in hemitropous seeds is given by prolongation of the hilar axis towards the root pole. On this line it can be discerned externally, sometimes even with the unaided eye, as a small tubercle or callosity (*E. odontocarpa*, Fig. 38*a*; *E. baileyana*), or it is hidden between ridges (*E. erythrocorys*, *E. tetradonta*) or wings (*E. tetragona*).

Inner and outer integument participate in its build-up, forming respectively the endostome and exostome. Due to the uniformity of the inner integument the endostome is of a relatively simple structure, whereas the exostome is bounded by three anatomically different parts (outer epidermis, integumentary parenchyma, crystal layer), of which each can contribute in various degrees to its formation. Thus, the micropyle as a whole exhibits in some species a very complex structure.

Reports upon the micropyle structure of mature seeds in general are rather scarce, owing probably to technical difficulties in obtaining suitable preparations. We have found the best way is to isolate in the micropylar region the inner cuticle, inner
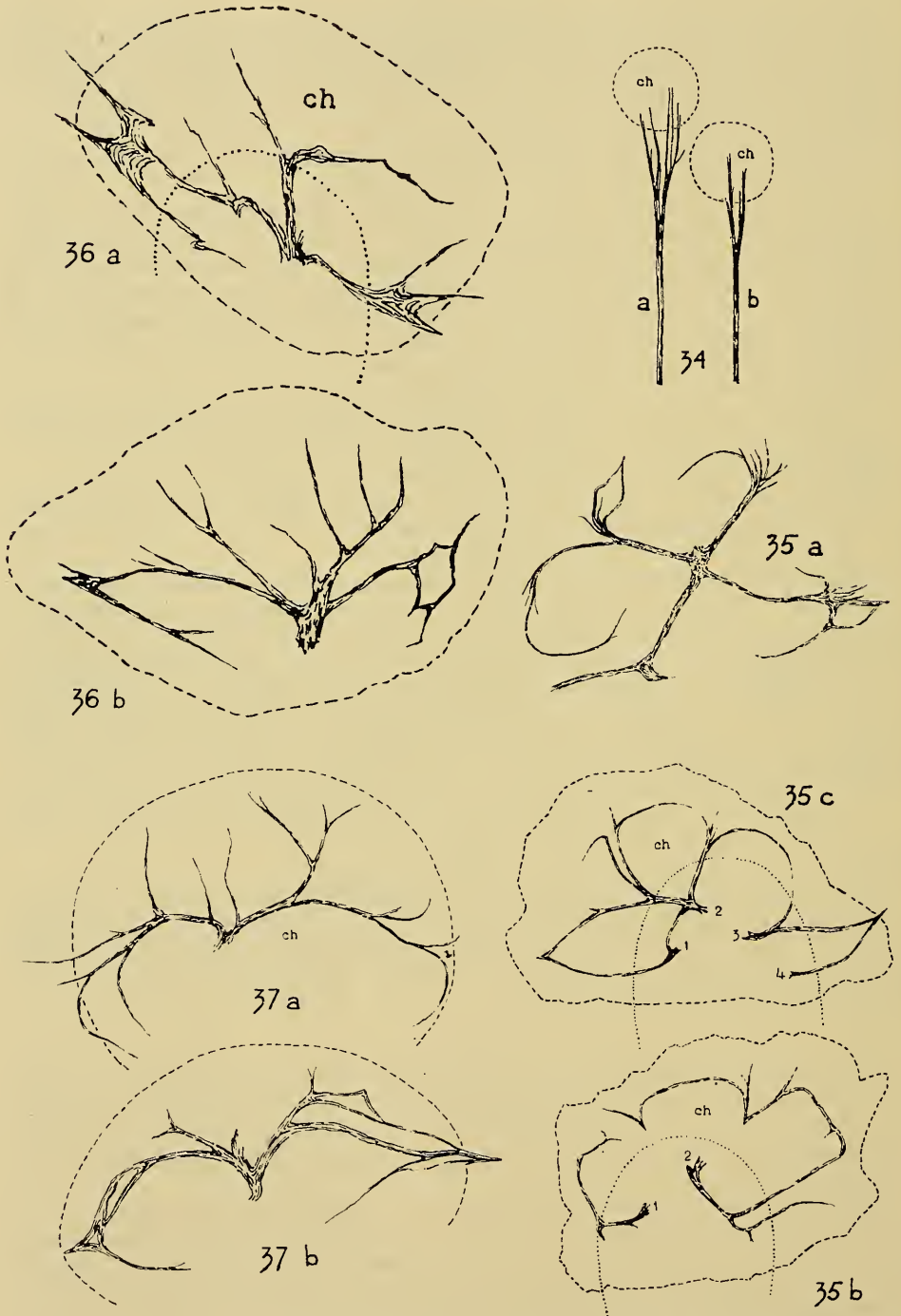
Text-fig. 34-37.—Vascularization patterns.   34, anatropous species (Leptospermae).   34*a*,
E. tenuipes, 85×; 34*b*, *E. curtisii*, 96×.   35-37, hemitropous species.   35*a-c*, *E. similis*, 45× (*a*
with one, *b* with two, *c* with four separately entering bundle strands) ; 36*a-b*, *E. lirata*, 96× ;
37*a-b*, *E. tetrodonta*, 45×.

(Broken line: chalaza extent; dotted line: hilum.)

integument, crystal layer and outer epidermis and to examine separately these layers in surface and side view.

Without claiming completeness some of our observations are illustrated in Text-figure 38 and on Plate xi. Variations, sometimes considerable, are frequent within the
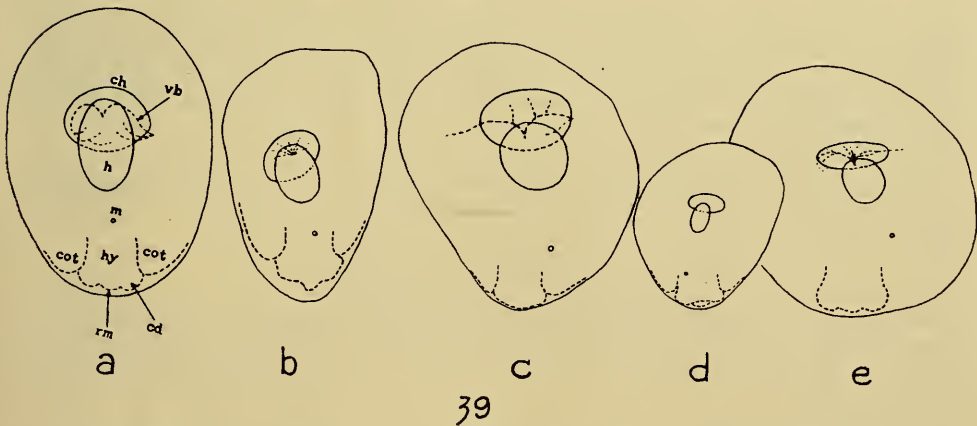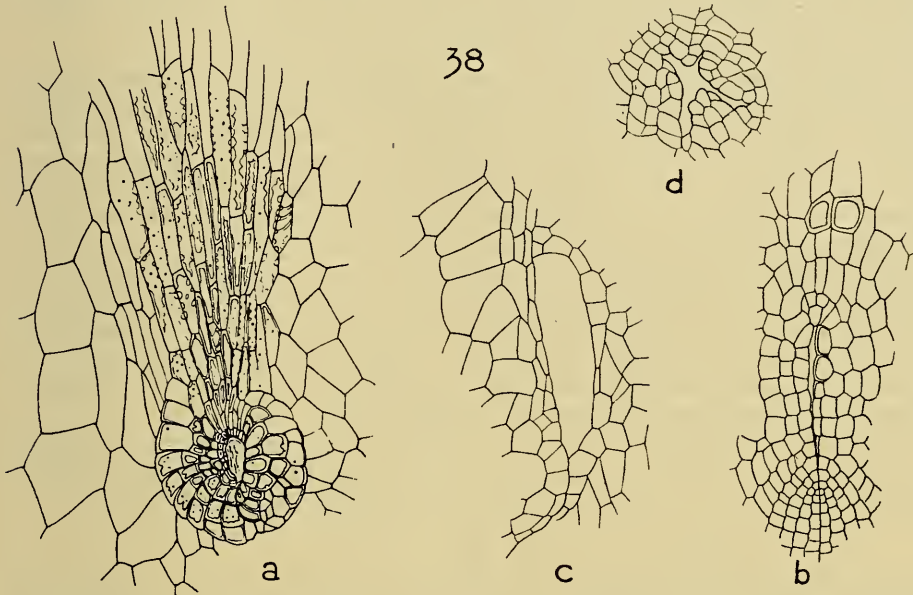
Text-fig. 38a-d.—Variations of the micropylar structure in *E. odontocarpa*, *ca.* 160×. *a*, surface view; *b*, *c*, exostome aperture in the (isolated) outer epidermis; *d*, endostome aperture (*d* was lying beneath *c*).

Text-fig. 39a-e.—Seed diagrams showing on the ventral side the relative position of chalaza (*ch*) with the vascular bundle (*vb*), the hilum (*h*), the micropyle (*m*), the hypocotyl (*hy*) with root meristem (*rm*) and clinging disc (*cd*), and the cotyledons (*cot*). *ca.* 9×.

a, *E. baileyana*; *b*, *E. odontocarpa*; *c*, *E. tetrodonta*; *d*, *E. ebbanoensis*; *e*, *E. eudesmoides*.

same species, especially with regard to the aperture of the micropyle. This is understandable if we bear in mind that the integuments have to keep pace with the increase in volume of the growing embryo, but there are differences in the rate of their growth which determine the final shape and size of the aperture in the integuments respectively in their different layers. On the seed surface for instance the micropyle may appear

as a circular or irregular opening (Pl. xi, fig. 2a), as a narrow or gaping slit (Fig. 38b, c), or it may be closed by contact or overlapping of its margins.

In separating the different layers in the micropylar region we first remove the endosperm which vaults gently into the suberized endostome (Pl. xi, fig. 1a) and is rich in oil and proteins. The inner cuticle is said in general to be apparently missing beneath the micropyle. But in *E. tenuipes* we could isolate it as a tender membrane with rib-like projections simulating a cellular structure (Pl. xi, fig. 3b).

The inner integument penetrates as a small or elongated hump (Pl. xi, fig. 1c, 2b), open or closed on the top, or as a two-lipped projection (the lips parted or firmly appressed) more or less deep into the exostome. In hemitropous seeds the inner limit of the exostome is well defined by the crystal layer which can be more or less vaulted, closed or with an open slit, the bounding cells of which are frequently devoid of crystals (Pl. xi, fig. 4b). We remember that in some species a crystal-free border of the crystal epithelium surrounds the chalaza.

In the exostome of *E. tetragona* the crystal layer is elongated into a tube or a horn-like protuberance which in *E. erythrocorys* can be up to 1 mm. long terminating just beneath the outer epidermis through which a capillary canal leads outwards (Pl. xi, fig. 1a). But in some instances we saw this "crystal horn" either as a blunt cone (Pl. xi, fig. 1b) or attenuated into a fine point and covered with integumentary parenchyma protruding freely between the epidermis cells.

It was said (Netolitzky, p. 38) that the disappearance of the cuticle lining the inner walls of the micropyle seems to be the rule. But in some species we saw it either well preserved (Pl. xi, fig. 1a, 2a, 4b) or in fragments (Pl. xi, fig. 4a).

Relative Position of Chalaza, Hilum, Micropyle and Hypocotyl.

In the hemitropous Eudesmiae the arrangement of these parts on the ventral side of the seed is illustrated by Figure 39. As can be seen, only in *E. baileyana* (Pl. xii, fig. 1; Text-fig. 39a) are they lying on the median line and symmetrical with it. In the other species they show considerable dislocations. Often the chalaza is turned away from the median and the hilum placed obliquely to it. As already mentioned, the position of the hilum determines always that of the micropyle: it lies on the prolongation of the longitudinal hilum axis towards the root pole.* The position of the hypocotyl with the apical root meristem indicates—and germination tests confirm—that it does not emerge through the micropyle.

In the anatropous Eudesmiae the disposition of those parts follows the Renantherae pattern (see Part I, Fig. 12); the chalaza lies on the raphe side just below the top. Hilum, micropyle and hypocotyl tip are at the basal end of the seed.

Nucellus, Endosperm and Embryo.

Remnants of the nucellus tissue, the cells empty and obliterated, are always present in variable amounts and extent and line the inner face of the testa. The endosperm remnants enveloping the embryo are better preserved. Their thick-walled cells are still rich in proteins.

Nucellus or endosperm (or both) project into the embryo folds between cotyledons and hypocotyl, thus showing ridges on the inner face. They may even penetrate into the wings (Fig. 28). The greatest amount is always found beneath the chalaza.

Generally, in the anatropous Eudesmiae the nucellus lines the whole inner face of the testa while the endosperm is restricted to the chalazal and micropylar region. In the hemitropous Eudesmiae the inverse relationship exists: the endosperm can be isolated as an unbroken layer enveloping the whole embryo whilst the nucellus is confined chiefly to the ventral side of the seed.

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* This direction is anatomically well marked by elongated rectangular epidermis cells leading from the base of the hilum to the micropyle (Text-fig. 29, 30; Pl. xi, fig. 5a; Pl. xii, fig. 1). Only a histogenetic investigation would reveal what significance has to be attributed to this constant feature.

The embryo, covered by a semi-solid cuticle, consists of the two cotyledons folded in a complicated way and bent down along the hypocotyl. Root and shoot are still at a primordial stage, that is, as apical meristems occupying the two poles of the hypocotyl which alone represents the embryo axis.

The reserve materials stored in the embryo are oil droplets and aleurone grains, the latter enclosing cluster crystals of calcium oxalate and globoids.

To exhibit the oxalate crystals it is advisable to remove successively the oil, proteins and globoids, whereupon they are clearly seen (up to 8 or more per cell). After treatment with HCl they disappear by formation of soluble $CaCl_2$, whereas cautious addition of sulphuric acid converts them on the spot into clusters of needle-shaped crystals of calcium sulphate.

The distribution of the cluster crystals of calcium oxalate in the embryo cotyledons is also worthy of note. In *E. erythrocorys*, *E. tetragona*, *E. eudesmoides*, *E. ebbanoensis* and *E. odontocarpa* they occur in all parts of the mesophyll, whereas in *E. baileyana* and *E. similis* the palisade tissue is devoid of them, though in the latter clusters in a few palisades can occasionally be observed. In *E. curtisii* and *E. tenuipes* (as well as in *Tristania conferta*) they seem to be missing altogether.

In Angophoras (*A. intermedia*, *A. costata*, *A. cordifolia*) they are confined to the upper epidermis, each cell containing one large druse or having instead a single crystal (tetragonal bipyramid?). Such crystals can be observed occasionally also among cluster crystals of *E. baileyana*, but here they are rare.

The globoids are generally of globular shape, but irregular form: even vermicular (*E. erythrocorys*) can be encountered frequently.

Is the Series Eudesmiae a Natural Group?

It is necessary to consider separately the Subseries with anatropous and hemitropous seeds in the light of the above results.

The two anatropous species, *E. tenuipes* and *E. curtisii*, agree completely in all anatomical details with each other, in spite of the quite different shape of their seeds. In *E. curtisii* they are very slender, "somewhat similar to those of *Tristania conferta* R.Br., and quite distinct from any other species of Eucalyptus". In *E. tenuipes* they are "D-shaped to oblique pyramidal" (Blakely, 1930). Thus, they are "leptosperm" in *E. curtisii* only, but of a "renantherous" type in *E. tenuipes*. Nevertheless they are undoubtedly close relatives, but the designation "Leptospermae" for this Subseries is inappropriate as it covers only one of the two species.

Among the hemitropous species the members of the Subseries Holocalyces: *E. baileyana*, *E. similis* and *E. lirata** seem also to form a natural group. Blake (*loc. cit.*, p. 335) mentioned that similarities of some vegetative and floral characters "suggest that *E. similis*, *E. baileyana* (and presumably *E. lirata*), *E. phoenicea* and *E. miniata* are better grouped together". We shall comment on this proposal after having investigated the Series Miniatae.

In the Subseries Efasciculares the seeds of its two species, *E. odontocarpa* and *E. tetrodonta*, have little in common both anatomically and morphologically, and to unite them in a group on seed characters alone appears impossible. *E. odontocarpa* has thin, flat, somewhat concave-convex seeds, carinate on the back, and with a circumferential wing which, though tender and narrow, has nevertheless a very complex structure (Fig. 28). The surface is wrinkled due to bulging mucilaginous epidermis cells, and the hilum sclerified (Pl. xii, fig. 1). In *E. tetrodonta* the seeds are angular-orbicular in outline and are depressed and rather thick, costate with the ribs built up by macrosclereids, the surface often warty due to groups of lignified cells perpendicularly elongated to it. There is no trace of sclerification in the hilum. Among the

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* Of *E. lirata* we had two seeds only (from the type specimen) for examination so that not all structural details could be investigated.

hemitropous Eudesmiae *E. odontocarpa* is the only species with a straight, palmately branched chalaza bundle (Pl. xii, fig. 1), whereas in *E. tetrodonta* the branches have an arctuous course (Fig. 37).   On the other hand, *E. tetrodonta* is the only species in the Eudesmiae without any suberization in the chalaza region.   Müller (Eucalypto-graphia, Dec. I) reports that *"E. tetrodonta* has no immediate close affinity to any of its congeners, except to *E. odontocarpa* . . .", and Blakely (Key, p. 70) says that "the two species are thrown together by the morphosis of the buds and fruits, but differing widely in habit".   Blake (*loc. cit.*, p. 335), commenting on Blakely's Eudesmiae, is cautious when suggesting that this Series "should perhaps be limited to *E. tetragona*, *E. odontocarpa*, *E. erythrocorys*, *E. eudesmoides*, and perhaps *E. tetrodonta*".

A still greater diversity is encountered in the Fasciculares, comprising *E. erythro-corys*, *E. tetragona*, *E. eudesmoides* and *E. ebbanoensis*.   *E. erythrocorys* has solid ribs formed by strongly elongated, thick walled, lignified epidermis cells, unique among the Eudesmiae (Fig. 22, 26).   *E. tetragona* and *E. eudesmoides* have winged seeds, those of *E. ebbanoensis* are without any testa expansion.   Thus, purely externally they all display a very different aspect.   Among the Eudesmiae *E. erythrocorys* is the only species with the rim of macrosclereids surrounding the hilum (Fig. 31) and with the most complicated reticular venation which in *E. tetragona* is very poor and simple (Pl. xiii, fig. 2).   Unique among all Eucalypts so far examined, and to our knowledge perhaps the Angiosperms hitherto investigated, is the suberization of the hilum in *E. ebbanoensis* (Pl. xi, fig. 5).   The micropyle structure of *E. erythrocorys* and *E. tetragona* is also unusual.   The crystal epithelium is elongated into a tube or horn-like protuberance, sometimes extended up to the outer epidermis (Pl. xi, fig. 1*a*), or even terminating freely between the epidermis cells (Pl. xi, fig. 1*b*).

We have stressed here only some of the more striking differences in morphological and anatomical seed characters of species allotted by Blakely to four Subseries and these united into the Series Eudesmiae.   In the light of the above evidence the question arises as to which facts induced Blakely to make this classification.   He considers (Key, p. 68) the Eudesmiae as "closely allied to Angophora particularly in the opposite character of the leaves, toothed calyx, texture of some of the fruits, and in the morphology of the seeds".

So far as the phyllotaxis and the calyx teeth are concerned, none of these characters is constant throughout the whole Series.

E. ebbanoensis, *E. similis* and *E. baileyana* are quoted as having alternate leaves. In *E. tetragona*, *E. tetrodonta*, *E. lirata*, *E. tenuipes* and *E. curtisii* opposite and alternate leaves occur.

With regard to the calyx teeth Blakely's designation of the Subseries "Holocalyces" expresses their lack in this group.

In view of the great variety of seed types in the Eudesmiae it is difficult to under-stand Blakely's hint at the morphological similarity of Angophora and Eucalyptus seed. Taking for instance the very different seeds of *E. erythrocorys*, *E. tetragona*, *E. ebbanoensis*, *E. baileyana*, *E. curtisii* or *E. tenuipes*; none resembles at all seeds of *Angophora intermedia*, *A. costata* or *A. cordifolia*.   A cursory glance at the anatomical structure of these Angophoras reveals a type of epidermis not seen in any Eudesmiae (thin-walled palisade-like cells rich in tannins), the inner integument completely or partially resorbed and not suberized, the oxalate druses of the cotyledons confined to the upper epidermis, etc.

Surprisingly, a comparison of *Eucalyptus curtisii* with *Tristania conferta* (both with anatropous seeds) reveals a perfect uniformity not only in all morphological details—as already pointed out by Blakely (Key, p. 72)—but also in the anatomical testa structure.

At this stage of our investigations further speculation about the affinity of species is not justified.

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EXPLANATION OF PLATES XI-XIII.

Plate xi.

(Cork tissue and cuticles brown.)

1-4: Micropylar structures.   1*a-c, E. erythrcorys*: 1*a,* longit. sect. (semi-diagrammatic), 45×; 1*b,* "crystal cone" protruding between epidermis cells (semi-diagrammatic), 45×; 1*c,* endostome from 1*a,* 125×.   2*a-b, E. ebbanoensis.* 330×: 2*a,* micropylar aperture in the outer epidermis; 2*b,* endostome (open).   3*a-b, E. tenuipes,* surface view, 250×: 3*a,* endostome (closed); 3*b,* inner cuticle beneath the endostome.   4*a-b, E. eudesmoides,* surface view, 250×: 4*a,* micropylar slit in the outer integument; 4*b,* micropylar slit in the crystal epithelium.   5*a-b, E. ebbanoensis,* hilum, 160×: 5*a,* surface view; 5*b,* trans. sect.

Plate xii.

Vascularization patterns (photomicrographs).

1: *E. odontocarpa, ca.* 100×.   *ch,* chalaza; *h,* hilum; *m,* micropyle.   2: *E. eudesmoides, ca.* 100×.

Plate xiii.

Vascularization patterns (photomicrographs).

1: *E. baileyana, ca.* 100×.   2: *E. tetragona, ca.* 90×.